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Review

Pre-mRNA splicing and retinitis pigmentosa

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Retinitis pigmentosa (RP) is a group of genetically and clinically heterogeneous retinal diseases and a common cause of blindness. Among the 12 autosomal dominant RP (adRP) genes identified, four encode ubiquitously expressed proteins involved in pre-mRNA splicing, demonstrating the important role that pre-mRNA splicing plays in the pathogenesis of retinal degeneration. This review focuses on recent progress in identifying adRP mutations in genes encoding pre-mRNA splicing factors and the potential underlying molecular mechanisms.

A major cause of blindness is retinal degeneration. One of the most common forms of retinal degeneration is retinitis pigmentosa (RP), affecting 1 in 4000 people worldwide and leaving more than 1.5 million people visually handicapped. First described over a hundred years ago, RP is characterized by progressive degeneration of the peripheral retina, leading to night blindness, loss of the peripheral visual field, and an abnormal electroretinogram. The most prominent pathological finding of RP is the loss of photoreceptor cells, often followed by alterations in the retinal pigmented epithelium and retinal glia with the appearance of bone spicule-like pigment deposits. RP can be both sporadic and familial. Genetic studies in the past several decades have significantly advanced our understanding of hereditary retinitis pigmentosa. Familial RP displays all three modes of Mendelian inheritance: autosomal dominant (adRP), autosomal recessive (arRP) and X-linked (xlRP) [1-8] and at the (RetNet) website and at the (Institute of Medical Genetics) at Cardiff, Wales website.

Genetic studies have identified over one hundred genetic loci associated with retinal diseases [3,6,9,10]. A number of genes defective in RP patients have been cloned. Many RP genes are expressed predominantly or specifically in the retina. Recently, four adRP genes were identified that are ubiquitously expressed in different tissues and associated with RNA processing. Three of these nonretina-specific adRP genes encode proteins essential for pre-mRNA splicing, pre-mRNA processing factors (PRPF), including PRPF31 (also named PRP31; for RP11) [10], PRPF8 (also named PRP8 or PRPC8; for RP13) [11] and PRPF3 (also named HPRP3; for RP18) [12]. Another RP gene, PAP1 (for RP9), has also been implicated in pre-mRNA splicing [13]. Among these, RP11 has been reported as the second most common locus for adRP, only after the rhodopsin gene [14]. However, the molecular mechanisms underlying the pathogenesis of RP caused by mutations in these broadly expressed genes are not well understood. The discovery of these ubiquitously expressed adRP genes, especially their involvement in pre-mRNA splicing, sheds new light on the pathogenetic mechanisms of retinal degeneration and may also impact on the design of therapeutic approaches to this photoreceptor disease. In this review, we will focus on recent progress on studying adRP caused by splicing factor mutations and discuss its implications.

Clinical and pathological features of retinitis pigmentosa: First described in the eighteenth century, RP encompasses a variety of retinal dystrophies, including both sporadic and hereditary forms. RP-like pigmentary deficits may also occur as a component of other clinical syndromes, such as Usher's syndrome [15].

In RP patients, the photoreceptor cell layer of the retina shows the first pathological signs of the disease. Typical RP, also called rod-cone dystrophy, involves both rod and cone photoreceptors. The rods are affected earlier and more severely than the cones. Following the loss of photoreceptor cells, the RPE undergoes thinning and atrophy in the peripheral retina, accompanied by attenuation of retinal arterioles. The degenerating RPE releases melanin pigment that accumulates in and around the walls of retinal vessels. These perivascular deposits are responsible for the characteristic "bone spicule pattern" as detected by funduscopic examination. In long-standing cases, gliosis and atrophy of the entire retina occur [16].

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The major clinical manifestations of typical RP are night blindness and tunnel vision early in the course of the disease, though visual acuity remains intact. As the disease progresses, the peripheral visual field is further reduced, and the cone photoreceptors are also affected, causing reduced visual acuity and impairments in color vision. Eventually, total blindness may ensue. In some atypical forms of retina pigmentosa called cone-rod dystrophy, the cones are affected first. Initially, cone-rod dystrophy manifests as loss of central vision. Later, the rods become affected, resulting in night blindness and the loss of peripheral vision, but some peripheral vision may be retained.

Retinal function tests help classify the clinical subtype of RP and assess the severity of the disease. Electroretinogram (ERG) and visual field tests can be used to stage and monitor the disease progression. The ERG measures the electrical response of the retina to various visual stimuli. The scoptopic (dark-adapted condition) tests the activity of rods and the photopic (light-adapted condition) tests the activity of cones. Clinically, ERG is useful in distinguishing rod-cone dystrophy from cone-rod dystrophy and in determining the severity of photoreceptor cell loss.

Genetic defects in retinal degeneration: Genetic studies on familial forms of retinal degeneration in the past twenty years have greatly advanced our understanding of retinal degeneration, although the majority of retinal disease cases are sporadic. The first locus for inherited photoreceptor degeneration (IPD) was identified in 1989 [17]. In 1990, rhodopsin was recognized as the first adRP gene with mutations in patients reported [18]. Progress in genetic studies of retinal degeneration has been well covered in several excellent reviews [6,19-21].

Among known genes associated with retinal degeneration, several groups are specifically expressed in the retina and are critical for retinal cell survival and function (for recent reviews see references [9,22]). The most commonly seen RP mutations occur in genes important for phototransduction. These genes include rhodopsin (RHO), the α- and β-subunits of rod cyclic guanosine monophosphate (cGMP) phosphodiesterases (PDE6A and PDE6B), the α-subunit of rod cyclic nucleotide gated channel (CNGA1) and arrestin (SAG). Mutations in genes important for visual cycles are also associated with photoreceptor cell degeneration. These include ATP-binding cassette transporter of rods (ABCR), cellular retinaldehyde binding protein (CRalBP), RPE 65 protein (RPE65) and RPE-retinal G-protein coupled receptor (RGR), all encoding proteins that are expressed mainly in RPE cells. These proteins are involved in recycling the rhodopsin chromophore 11-cis-retinaldehyde, which absorbs light to initiate the phototransduction cascade. Another group of genes defective in retinal degeneration code for photoreceptor cell structural proteins, including peripherin (also named retinal degeneration slow, RDS), rod cell outer membrane protein 1 (ROM1), retinal actin-binding protein fascin (FSCN2) and prominin like-1 (PROM-1). In addition, genes encoding critical metabolic enzymes, such as inosine monophosphate dehydrogenase 1 (IMPDH1) and lecithin retinol acyltransferase (LRAT), are also important for retinal function, and mutations in these genes cause retinal degeneration.

Another category of genes associated with retinal degeneration are those encoding proteins regulating gene expression at the transcriptional or posttranscriptional levels. Mutations in genes encoding transcription factors and posttranscriptional RNA processing factors have been identified in retinal degeneration patients. Interestingly, all known transcription factors associated with retinal degeneration are photoreceptor-specific transcription factors, including nuclear receptor NR2E3, neural retina leucine zipper (NRL) and cone-rod homeobox (CRX). On the other hand, four adRP genes encoding pre-mRNA splicing factors are all ubiquitously expressed. It is also worth noting that among the 12 adRP genes cloned, four of them encode pre-mRNA splicing factors or proteins interacting with splicing factors (RetNet website: RetNet; Table 1), clearly indicating the important role of pre-mRNA splicing in the pathogenesis of retinal degeneration.

Pre-mRNA splicing and splicing machinery assembly: Pre-mRNA splicing is a critical step in mammalian gene expression. The vast majority of mammalian transcription units contain noncoding intervening sequences (IVS, also called introns) that must be accurately excised to form functional messenger RNA (mRNA). Pre-mRNA splicing occurs via a two-step transesterification mechanism. The first step is the cleavage at the 5' splice site (5' SS) and the formation of the lariat intermediate. The second step is the cleavage at the 3' splice site (3' SS) with concomitant ligation of the 5' and 3' exons and subsequent degradation of the lariat intermediate (Figure 1). These biochemical reactions of pre-mRNA splicing reaction take place in spliceosomes, which are large RNA-protein complexes containing pre-mRNA, five small nuclear ribonucleoprotein (snRNP) particles, U1, U2, U4/U6 and U5, as well as more than a hundred accessory protein factors [23-27].

Spliceosome assembly is a dynamic process, involving multiple RNA-RNA, RNA-protein and protein-protein interactions. During spliceosomal assembly and activation, there are a series of conformational rearrangements. This process involves not only changes in the RNA conformation but also remodeling of snRNPs and exchanges of protein factors (for example, see reference [28]). At the catalytic center of the functionally mature spliceosome, the 5' SS and 3' SS must be juxtaposed precisely to ensure accurate cleavage and ligation of exons. Spliceosome assembly involves step-wise interactions between different spliceosomal UsnRNPs and the pre-mRNA substrate. Immediately after transcription, the nascent pre-mRNA transcript is committed to splicing pathway. The 5' splice site is initially recognized by U1 snRNP, while the branch site sequence interacts with U2snRNP. The stable association of U2snRNP with the branch site is ATP-dependent and involves additional accessory protein factors. The next ATP-dependent step is the incorporation of U4/U6.U5 tri-snRNP complex into the spliceosome with the dissociation of U1snRNP from the 5' splice site. The 5' SS sequence is then engaged in interactions with U6snRNA around the intronic region and with U5 snRNA at the exonic region. After escort-

ing the U6snRNP into the splicing complex, the U4snRNP is released. Following extensive rearrangement, the catalytically active functional spliceosome is formed, in which the cleavage and ligation reactions occur to remove the intron (Figure 2).

The recruitment of the U4/U6.U5 tri-snRNP complex into the spliceosome is a critical step in spliceosome assembly. U6 snRNP plays an important role at the catalytic center of the spliceosome. The base-pairing interactions between U4 snRNA and U6 snRNA lead to the formation of the U4/U6 snRNP, which associates with U5snRNP as the U4/U6.U5 tri-snRNP complex to join the spliceosome. The formation of U4/U6.U5 tri-snRNP complex and the association of tri-snRNP with the spliceosome require a number of protein factors, including PRPF8 (also named U5-220 kDa), PRPF31 and PRPF3 (Figure 2; also next section). These proteins are highly conserved through evolution, from yeast to human [23,26,27,29].

Pre-mRNA splicing and neurodegenerative diseases: It has been estimated that 15% of point mutations that cause human genetic diseases affect pre-mRNA splicing [30]. Because most genetic studies focus on exonic regions, this is likely to be an underestimate. Splicing mutations can be classified into two categories: cis-acting mutations that affect pre-mRNA splicing of the corresponding genes and trans-acting mutations that cause defective splicing machinery or splicing regulators, which in turn lead to aberrant pre-mRNA splicing of target genes involved.

A large number of cis-acting splicing mutations have been found in neurodegenerative diseases (such as tauopathies including Alzheimer's disease), malignant diseases (such as retinoblastoma) and other neurological disorders including retinal degeneration [27,31-33]. Mutations can occur in exonic regions, exon-intron boundaries or intronic regions. Some mutations are missense or nonsense mutations because of nucleotide changes in exonic regions or at the exon-intron boundaries, and other mutations result in deletion or truncation of the polypeptide encoded because of exon skipping or intron inclusion. Such splicing mutations are associated with a wide range of human diseases affecting different tissues and organs, including the retina. Splice site mutations have been reported in different types of RP. Multiple splice site mutations in rhodopsin genes have been documented in adRP pa-

Table 1. Genes identified in autosomal dominant retinitis pigmentosa

Gene Name	Protein function	Expression pattern	RP locus	Chromosomal location	References
RHO	Rhodopsin, phototransduction	Photoreceptor-specific	RP4	3q21-q24	[18]
RDS	Peripherin/RDS, photoreceptor structural protein	Photoreceptor-specific	RP7	6p21.1-cen	[96]
ROM1	Retinal outer segment membrane protein 1, Photoreceptor structural protein	Photoreceptor-specific		11q13	[97]
FSCN2	Fascin2, crosslinking and bundling F-actin	Photoreceptor-specific		17q25	[78]
CRX	cone-rod otx-like homeobox transcription factor	Photoreceptor-specific		19q13.3	[60]
NRL	neural retina leucine zipper transcription factor	Photoreceptor-specific	RP27	14q11.2	[98]
RP1	A protein with similarity to doublecortin, microtubule-associated protein	Photoreceptor-specific	RP1	8q11-q13	[99-101]
IMPDH1	Inosine monoposphate dehydrogenase 1, catalyzing the rate-limiting step in guanine synthesis	A range of tissues including retina, lung, thymus, and brain	RP10	7q31.1	[102,103]
PRPF31	Pre-mRNA splicing factor	ubiquitous	RP11	19q13.4	[10]
HPRP3	Pre-mRNA splicing factor	ubiquitous	RP18	1q21.1	[12]
PRPC8	Pre-mRNA splicing factor	ubiquitous	RP13	17p13.3	[11]
PAP1	Associated with HPRP3, possible pre-mRNA splicing factor	ubiquitous	RP9	7p15.1-p13	[14,85,88]

tients [34,35]. A recent example in RP patients is the defective splicing caused by splice site mutation in intron 3 of rhodopsin pre-mRNA [36]. Splice site mutations also affect the function of other retinal genes and lead to RP. Splice site mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene cause xlRP [37]. A splice site mutation in intron 2 of beta-subunit of cGMP phosphodiesterase gene causes defective pre-mRNA splicing and leads to arRP [38].

The pathogenic effects of transacting mutations that disrupt pre-mRNA splicing machinery or splicing regulation have only been recognized recently. Although the genes mutated are ubiquitously expressed, their mutations cause cell-type specific manifestations. One example is spinal muscular atrophy (SMA), a motor neuron specific disorder caused by deletion of, or mutations in, Survival of Motor Neurons (SMN), a ubiquitously expressed gene that is important for splicing machinery assembly [39]. An example of transacting mutations that affect splicing regulators is myotonic dystrophy (DM), a common form of adult-onset muscular dystrophy. Type 1 and type 2 myotonic dystrophy are caused by CTG expansion in the 3' untranslated region of DM protein kinase (DMPK) gene and by CCTG expansion in intron 1 of ZNF9 gene, respectively. Accumulating evidence supports a gain-of-function for RNA transcripts of the mutant alleles in these DM patients, leading to disruption of pre-mRNA splicing regulation as a result of dysfunction of CUG-binding proteins (for review see reference [33]). Another example of diseases associated with transacting mutations is autosomal dominant oculopharyngeal

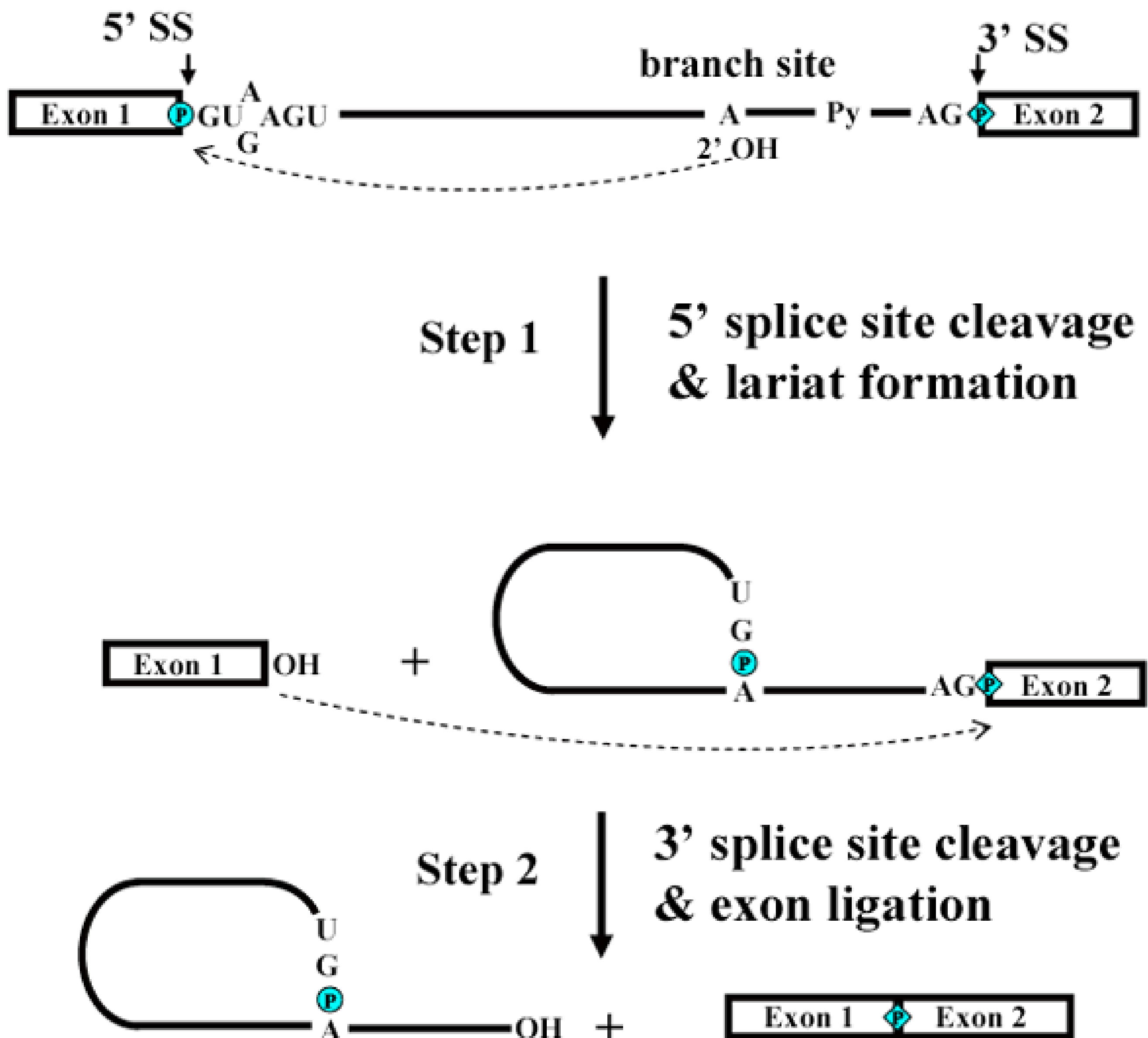

Figure 1. A two-step mechanism of pre-mRNA splicing. Biochemical mechanism of pre-mRNA splicing. Pre-mRNA splicing occurs via a two-step transesterification mechanism. The phosphodiester linkages are indicated by the letter p inside a circle or a diamond [27].

muscular dystrophy (adOPMD), characterized by ptosis, muscle weakness, and dysphagia. Recent studies have revealed short (GCG) 8-13 triplet-repeat expansions in the polyadenylation binding protein 2 (PABP2) gene in adOPMD patients [40,41], suggesting a potential involvement of RNA processing defects. The recent discovery of adRP mutations in genes encoding essential splicing factors clearly demonstrates the important role of such transacting mutations in the pathogenesis of retinal degeneration.

Pre-mRNA splicing and retinitis pigmentosa: It is remarkable that four out of 12 cloned adRP genes are ubiquitously expressed genes associated with pre-mRNA splicing (Table 1). Three of these genes encode essential spliceosomal proteins that are critical for the formation of the mature splicing complex, PRPF31, PRPF8, and PRPF3. A fourth gene, PAP1, is also implicated in pre-mRNA splicing and has been shown to interact with PRPF3. Biochemical and genetic studies demonstrate that PRPF8, PRPF31, and PRPF3 are required for the formation of stable U4/U6 snRNPs and for assembly of the U4/U6.U5 tri-snRNP. It has been proposed that PRPF3 and PRPF31 are involved in activation of the spliceosome by unwinding of the U4/U6 duplex [42]. PRPF8 is crucial for the

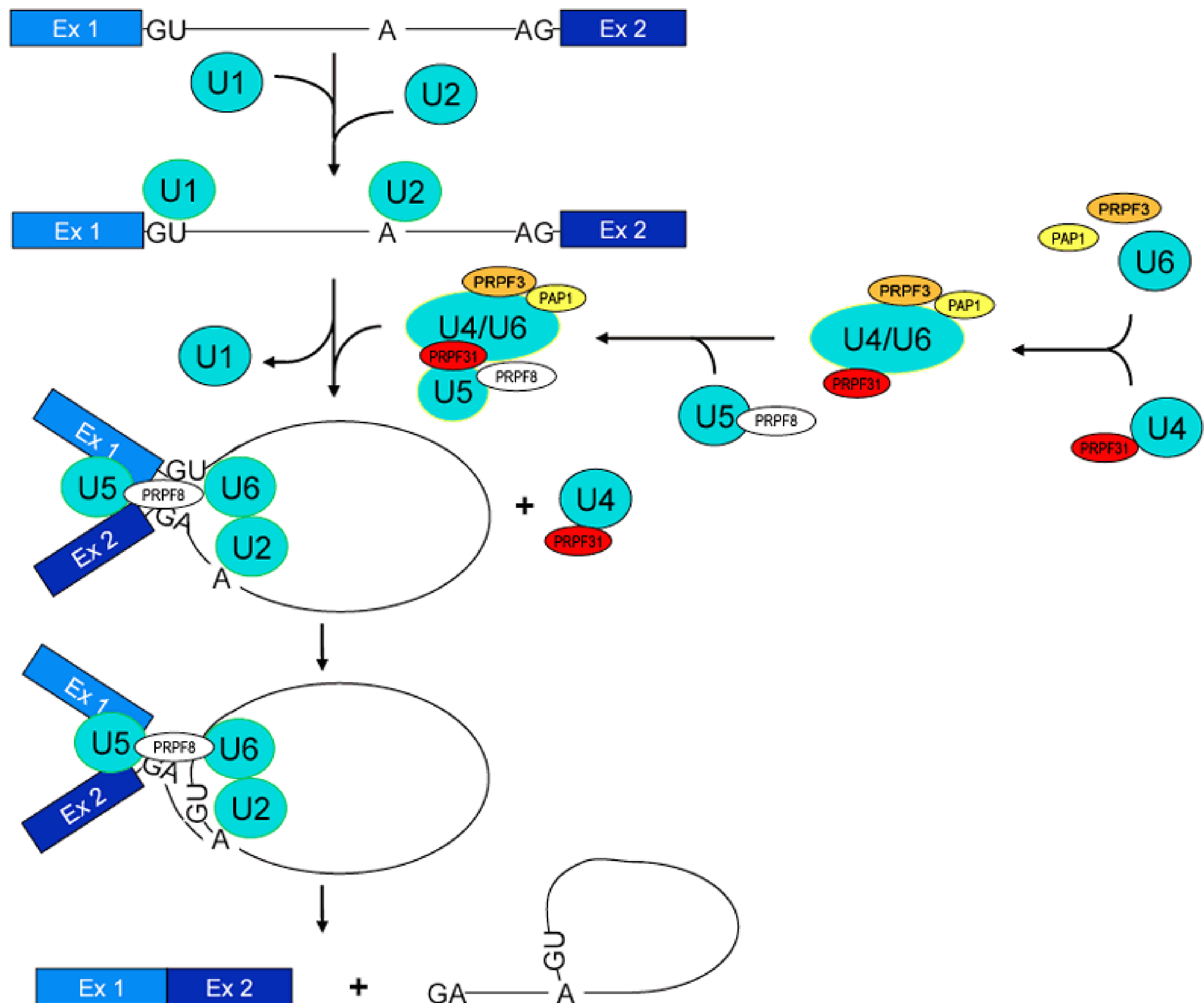

Figure 2. Spliceosome assembly and the involvement of adRP associated splicing factors. During the early step of spliceosome assembly, the U1snRNP and the U2snRNP interact with the conserved sequences at the 5' splice site (GU) and the branch point adenosine (A) of the 3' splice site, respectively, to form the prespliceosome. Next, the U4/U6 snRNP associates with the U5snRNP to form the U4/U6.U5 tri-snRNP, which is incorporated into the spliceosome. The formation of the U4/U6.U5 tri-snRNP complex requires PRPF8, PRPF3 and PRPF31, among other splicing factors. U1 is displaced from the 5' splice site, and U4 dissociates from U4/U6 snRNP, allowing U6 to interact with the 5' splice site and with the U2snRNP. In the mature spliceosome, splice sites are aligned in the catalytic center, and the two sequential trans-esterification reactions occur (Figure 1), resulting in the removal of the intron [95]. (Adapted by permission from Macmillan Publishers Ltd: Patel AA, Steitz JA. Splicing double: insights from the second spliceosome. Nature Reviews Molecular Cell Biology 4: 960-970, 2003)

formation of the catalytic center in the spliceosome. All four adRP-related splicing genes are involved in the formation of the splicing machinery.

RP11 and the PRPF31 gene: RP11 was first identified by linkage analysis in a large British adRP family and mapped to chromosome 19q13.4 [43,44]. This was followed by the discovery of other British as well as European and Asian families. Most of the RP11 pedigrees showed the type II/regional form of RP [14,43-47]. Screening of the affected members of RP11-linked families led to the identification of mutations in the PRPF31 gene. Human PRPF31 gene spans about 18 Kb and contains 14 exons [10]. The PRPF31 mutations include missense substitutions, deletions, nonsense, and insertions and have been identified in RP11-linked families and sporadic RP11 cases (Table 2) [10,47-49]. A 12 bp deletion in the PRPF31 gene was identified in 19 individuals in a large Chi-

Table 2. Pre-mRNA splicing factors and the mutations that caused autosomal dominant retinitis pigmentosa

Name	Chromosome	Mutation sites	Mutations
PRPF31 (PRP31, RP11)	19q13.33-13.43	Exon 5	12 bp deletion, (H111K112F113I114)
		Intron 5	IVS5-1G to A, aberrant splicing
		Exon 6	1 bp deletion, frame-shift
		Intron 6	RP677; IVS6-3 to -45 deletion; aberrant splicing
		Intron 6	RP1907; IVS6+3A to G; aberrant splicing
		Exon 7	AD29; 646G to C, A216P
		Exon 7	SP14; 33 bp duplication at nucleotide 580-581; 11 aa inframe insertion
		Exon 7	SP42; 581C to A, A194E
		Exon 8	SP117; 1 bp insertion at 769-770; frameshift after amino acid residue 256
		Exon 11	AD5; 11 bp deletion, frameshift after amino acid residue 371
PRPF8, (PRPC8, PRP8, RP13)	17p13.3	Exon 42	6942C to A, P2301T
		Exon 42	6953C to G, F2304L
		Exon 42	6967A to C, H2309P
		Exon 42	6967A to G, H2309R
		Exon 42	6969A to G, R2310G
		Exon 42	6970G to A, R2310K
		Exon 42	6983C to A, F2314L
		Exon 42	6 bp deletion with 11 bp insertion at nucleotides 6972 to 6977, frameshift
PRPF3 (HPRP3, PRP3 RP18)	1p13-q21	Exon 11	1478 C to T, P493S
		Exon 11	1482 C to T, T494M
PAP1 (RP9)	7p14.2	Exon 5	410A to T, H137L
		Exon 6	509A to G, D170G

Pre-mRNA splicing factor genes that are associated with adRP are listed, including pre-mRNA processing factor (PRPF) PRPF31, PRPF8, PRPF3, and PAP1 (Pim-1 kinase associated protein 1). Their chromosomal localization and mutations identified in RP patients are described.

nese family with RP [48]. The 12 bp deletion is located in exon 5 of PRPF31 gene and results in an in-frame deletion of four amino acids: $H_{111}K_{112}F_{113}I_{114}$. The H_{111} residue is highly conserved throughout evolution among different species. A single nucleotide change (G to A) at the position -1 of intron 5 of the PRPF31 gene was found in another Chinese pedigree [47]. This changes the consensus AG dinucleotide sequence of the intron 5 splice acceptor to AA. This splicing site mutation has been predicted to cause aberrant splicing of the gene, either intron 5 retention or exon 6 skipping [47]. In family AD29, a missense A216P mutation was found in this highly conserved amino acid residue [10]. In family AD5, an 11 bp deletion (1115-1125 del) in exon 11 results in an aberrant truncated protein of 469 residues. Thus, mutations from RP11-affected families have been detected in exon 5, 6, 7, 8, 11 along the PRPF31 coding region as well as in the intron regions of the PRPF31 gene (Table 2).

A unique feature of RP11 is the bimodal expressivity of the mutations in the PRPF31 gene. Individuals carrying mutations in the PRPF31 gene can either be completely asymptomatic or manifest severe phenotypes at early ages. Asymptomatic carriers can have both affected parents and affected children [50,51]. Symptomatic individuals show early onset night blindness and vision loss, often in their teenage years and typically become blind by their early thirties [44]. Measurements of the mRNA level of PRPF31 from lymphoblastoid cell lines derived from an adRP family suggests a correlation of the expression level of the wild-type allele with the pheno-

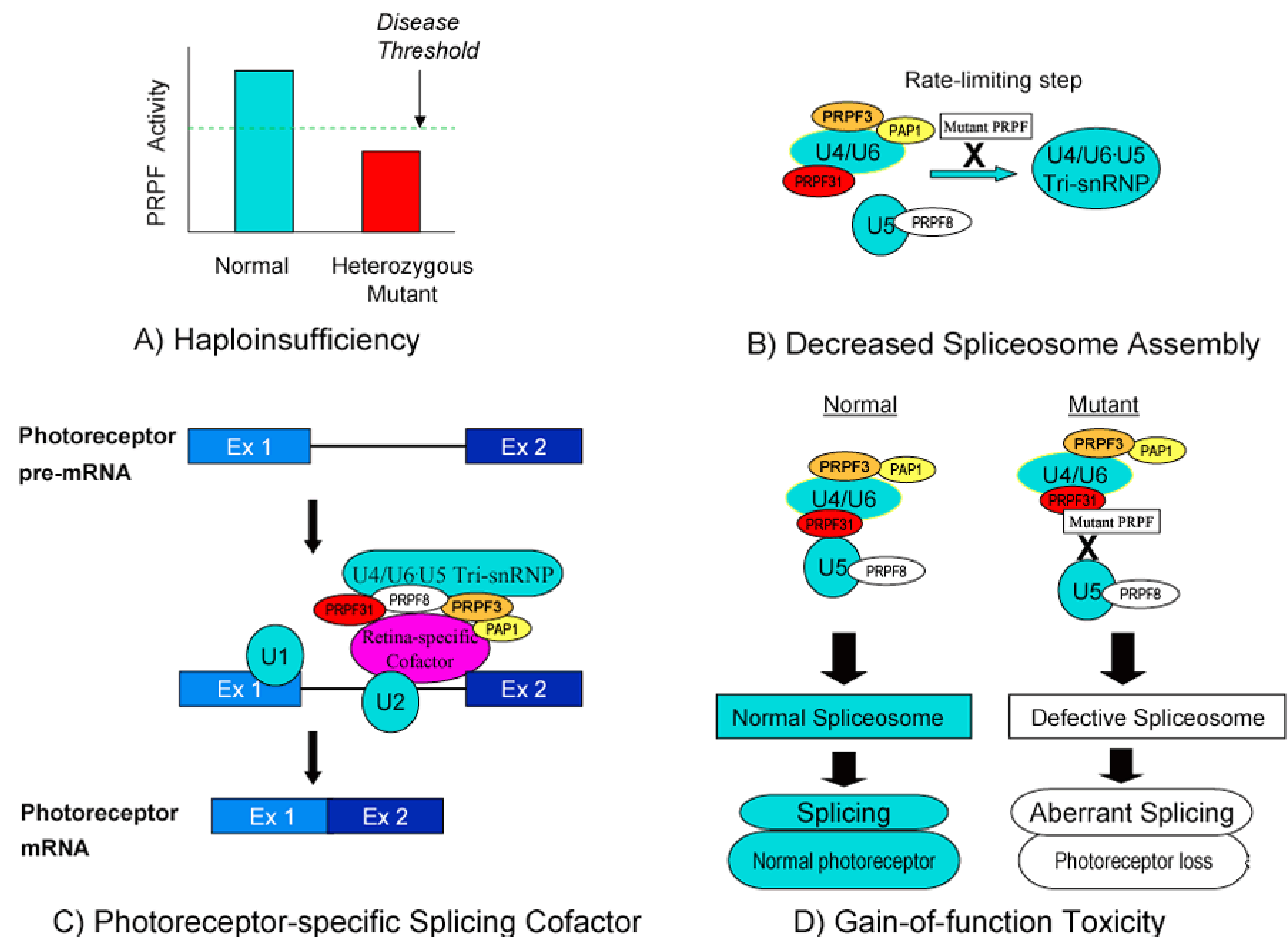

Figure 3. Proposed models of the pathogenesis of adRP associated splicing factor mutations. **A**: A general haploinsufficiency model: a threshold level of splicing factors is essential for normal photoreceptor cell function. A mutant allele for adRP associated splicing factors may result in the expression of a nonfunctional protein. A decrease below the threshold level to support pre-mRNA splicing in photoreceptor cells leads to the development of RP. **B**: A more specific sub-model of Model A in which tri-snRNP assembly is rate-limiting only in photoreceptor cells. A mutant allele may result in the expression of a functional, but sub-optimal, splicing factor. Because of the extremely high metabolic demands of photoreceptor cells, a slight decrease in splicing efficiency could result in cell death. **C**: A photoreceptor-specific splicing factor model. The adRP associated splicing factors functionally interact with an unidentified photoreceptor-specific factor essential for pre-mRNA splicing in photoreceptor cells. AdRP mutations may disrupt the interaction with such retina-specific cofactor, resulting in defective splicing of critical photoreceptor genes. **D**: A gain-of-function toxicity model. AdRP mutant proteins can block the function of wild-type alleles, leading to aberrant pre-mRNA splicing.

type, although the expression level of the mutant protein was not determined [52]. It is possible that genetic modifier genes may influence the expression of PRPF31 mutations. Consistent with this, asymptomatic patients inherit a wild-type allele different from the one inherited by their symptomatic siblings [10]. The penetrance of the PRPF31 mutations may be influenced in trans by silent PRPF31 alleles or by a closely linked locus on the wild-type chromosome, as suggested by McGee et al. [46].

The human PRPF31 gene encodes a 499 amino acid polypeptide. It is a 61 kDa U4/U6 snRNP protein, homologous to *Saccharomyces cerevisiae* pre-mRNA splicing factor Prp31p. The yeast prp31 gene was identified in a screen of temperature-sensitive mutants for splicing defects upon a shift to non-permissive temperatures. It was shown to be essential for pre-mRNA splicing and cell viability. Deletion of the prp31 gene inhibits tri-snRNP assembly and blocks pre-mRNA splicing [53,54]. Expression of the mammalian PRPF31 gene has been detected in a variety of tissues from kidney, lung, heart and brain to retina [10,55]. PRPF31 contains a conserved Nop domain, which is a putative snoRNA binding domain that mediates protein-RNA interactions in RNA processing [56,57]. PRPF31 interacts with U4 snRNA (Nottrott, 2002 [42]) and is required for the U4, U5/U6 tri-snRNP assembly [53,54,58]. In HeLa cells treated with siRNA to knock-down PRPF31, tri-snRNP formation was inhibited with the accumulation of U5 mono-snRNPs and U4/U6 di-snRNPs, demonstrating the essential role of PRPF31 in tri-snRNP assembly [59]. Biochemical experiments have shown that PRPF31 interacts directly with a 102 kDa U5 snRNP protein. This indicates that it may tether the U5 snRNP to the U4/U6 snRNP to form the tri-snRNP, thus promoting the recruitment of the tri-snRNP into the spliceosome and facilitating the formation of the catalytically active spliceosome [58].

RP13 and the PRPF8 gene: RP13 was first identified by linkage mapping in a large South African family [36] and later confirmed by cases from other countries [11]. The affected individuals showed the classical RP phenotype of early onset night blindness and severe visual field loss. The RP13 gene was cloned, and mutations were identified in the PRPF8 gene [11]. Patients with these PRPF8 mutations develop extensive retinal degeneration and visual disability by middle age [11,36,60]. When retina samples of a patient carrying a PRPF8 mutation, Arg2310Gly, were compared with retina with mutations in the rhodopsin gene (Pro23His, Cys110Arg, and Glu181Lys), similar histologic changes were found, suggesting a final common pathway leading to photoreceptor loss [61]. So far, all of the PRPF8 mutations identified in RP13 patients are missense mutations or deletion/insertions, clustered in the conserved amino acid residues in the last exon, exon 42 (Table 2) [11,60,62]. These observations suggest the importance of this region in PRPF8 function in photoreceptor cells.

PRPF8 is the human ortholog of the yeast splicing factor Prp8p. It is a 220 kDa U5 snRNP core protein that is very highly conserved through evolution with 62% sequence identity between the yeast and human proteins [63]. The human and yeast PRPF8 proteins are likely to play a similar role in pre-mRNA splicing. Yeast Prp8p interacts via its carboxyl terminus with another U5 snRNP protein, a RNA helicase named Brr2p that unwinds U5/U6 helices [64]. PRPF8 is required for the formation of the U4/U6-U5 tri-snRNP [65]. Accumulating evidence supports that PRPF8 acts at the catalytic center of the spliceosome and coordinates multiple processes during spliceosome activation [66]. PRPF8 interacts with spliceosomal snRNAs (U5 and U6 snRNAs) and with pre-mRNA at all major splicing signals, including 5' splice site, branch sites and 3' splice sites [67-73]. PRPF8 mediates interactions among multiple spliceosomal components including snRNAs and proteins, playing critical roles in the formation of the spliceosomal catalytic center and in the alignment of the 5' and 3' splice sites at the catalytic center [64,69-75]. However, the precise activity of PRPF8 in pre-mRNA splicing and the mechanisms underlying the pathogenesis of PRPF8 mutations remain unclear.

RP18 and the PRPF3 gene: The *RP18* locus was mapped to a <2 centimorgan (cM) interval between markers D1S442 and D1S2858 in a large four-generation Danish family on chromosome 1q21.1 [76,77]. These patients showed night blindness at the end of the first decade of life with relatively slow progression of visual field loss during the third and fourth decade of life while the macular area remained relatively spared. Some eventually became totally blind at a late stage [76]. Sequence analyses revealed two missense mutations in the PRPF3 (HPRP3) gene in RP18-linked families (Thr494Met and Pro493Ser in the 11th exon, Table 1) [12]. T494M is found repeatedly in unlinked families from England, Demark, Spain, and Japan, indicating the possibility of a mutation hot spot [12,49,78]. These mutated amino acid residues are highly conserved in all known *PRPF3* orthologues, suggesting an important function of this region of PRPF3 in the pre-mRNA splicing process [12].

Human PRPF3 contains 682 amino acids and is a 77 kDa protein homologous to yeast Prp3p [79,80]. The protein sequence analyses of PRPF3 polypeptide from different species indicate that their C-terminal sequences are highly conserved, whereas their N-terminal sequences are not. This suggests a conserved function for the C-terminal end of this splicing factor in pre-mRNA splicing through evolution [12].

PRPF3 is also a U4/U6 snRNP-specific splicing factor whose yeast homolog had been identified through genetic screening. Yeast Prp3 mutations cause instability of the U4/U6 snRNP and inhibit tri-snRNP formation [81]. PRPF3 protein is tightly associated with the U4/U6 snRNP [79]. PRPF3 interacts with another U4/U6 snRNP protein, PRPF4. PRPF3 and PRPF4 can be co-purified from HeLa cells together with the 20 kDa cyclophilin H as a heterotrimer protein complex. The central domain (amino acids 195-443) of the PRPF3 protein plays a key role in the formation of PRPF3-PRPF4 complex [82]. PRPF3 is required for the assembly and activation of the spliceosome. PRPF3 protein may play a role in the recruitment of other factors for U4/U6 snRNP assembly. However, its specific involvement in this process is still not clear [82,83].

RP9 and the PAP1 gene: RP9 was first mapped to a locus on human chromosome 7p14 by analysis of a nine generation adRP pedigree from England [84]. Recently, PAP1 was implicated as the defective gene in RP9 [85]. PAP1 is expressed in a wide range of tissues, and its precise function is not known. The *PAP1* gene encodes a putative nuclear localization signal and a carboxyl terminal domain rich in lysine and serine. The carboxyl terminal domain is phosphorylated by Pim-1 kinase in vitro [86]. Pim-1 is an oncogene encoding a serine/threonine protein kinase, which is involved in suppression of apoptosis and cell proliferation [87]. PAP1 is colocalized in a speckle pattern in the nucleus with a splicing factor of the SR family, SC35. Its C-terminal lysine-rich region, amino acids 168 to 175, is believed to mediate this colocalization [13]. PAP1 is associated with the U4/U6.U5-tri-snRNP complex [88]. PAP1 interacts with proteins involved in splicing. PAP1 interaction partners include PRPF3 and a SR domain containing protein, CBF1-interacting transcription corepressor, CIR [88,89]. CIR influences splicing regulation and interacts with SR proteins, SC35, ASF/SF2 as well as U2AF35. These proteins play an important role in early steps of spliceosome assembly.

RP9 is a rare disease. Only two missense mutations in PAP1 have been described, H137L, D170G, in adRP patients [85]. Interestingly, individuals carrying typically pathogenic mutations at the RP9 locus can also be asymptomatic [90].

Molecular mechanisms underlying retinitis pigmentosa mutations in pre-mRNA splicing factors: The molecular mechanisms by which mutations in ubiquitously expressed splicing factors cause photoreceptor-specific disease remain unclear. The discovery of adRP mutations in these pre-mRNA splicing factor genes clearly demonstrates the importance of pre-mRNA splicing in photoreceptor survival and function. It is likely that the pre-mRNA splicing of certain critical photoreceptor genes depends on the expression of both copies of wild-type genes. Either loss of function or gain-of-function mechanisms may lead to defective RNA processing in photoreceptor cells.

Several models can be proposed to explain the photoreceptor-specific phenotype (Figure 3). The first is the haploinsufficiency model. In this model, the loss of one functional copy of these essential splicing genes leads to insufficient function in supporting rod photoreceptors because of their high demand for mRNA expression and protein synthesis. Rod outer segments undergo continuous renewal through disk morphogenesis and shedding [91]. Indeed, mice heterozygous for the rhodopsin gene, therefore producing only 50% of the normal level of rhodopsin protein, show retinal degeneration. It is possible that mutations in PRPF3, PRPF8 PRPF31 and PAP1 genes may lead to reduced levels of wild-type functional proteins, insufficient to maintain the level of RNA processing required for normal function and survival of photoreceptor cells. Consistent with this model is the observation that the expression levels of PRPF31 in lymphoblastoid cell lines derived from patients and asymptomatic mutation carriers correlates with phenotypes [52]. However, direct experimental evidence supporting this model is still missing.

A related model is that the adRP-associated mutations may decrease the rate at which spliceosome activation occurs [75], making pre-mRNA splicing a rate-limiting step in photoreceptors but not other cells. A common feature shared by the adRP-associated splicing factors, PRPF8, PRPF31 and PRPF3, is that they are all involved in recruiting the U4/U6-U5 tri-snRNP to form the catalytically active spliceosome. PAP1 is also implicated in this step of spliceosomal assembly.

The third model is that the presence of photoreceptor-specific cofactor(s) that act as common essential partner(s) for these adRP-associated splicing factors causes the photoreceptor-specific manifestations. So far, only a limited number of known RNA binding proteins are neuronal specific. For example, Nova proteins, RNA binding proteins with hnRNP K homology type, have been implicated in neuronal specific alternative splicing. Nova1 knock-out mice exhibit changes in alternative splicing of glycine receptor a2 and GABA receptor g2 [92]. To date, no photoreceptor-specific RNA binding proteins or splicing factors have been reported.

The fourth model is that the production of mutant splicing factors causes dominant negative, gain-of-function toxicity to photoreceptor cells but not in other cell types. Several lines of evidence support this mechanism. In RP13 patients, all of the identified mutations are missense ones. Together with the severe phenotype of RP13, these findings suggest a possible dominant-negative mechanism in PRPF8-associated adRP [11]. In RP11 cases, many mutations in PRPF31 gene, including insertion, deletion, and splice site mutations, are predicted to cause protein truncation [10]. In addition to these observations, in vivo experiments also support this gain-of-function mechanism. The overexpresssion of AD5 or SP117 mutant PRPF31 proteins in primary retinal cells causes a significant increase in the death of rhodopsin-positive retinal cells. Co-expression of these RP mutant PRPF31 proteins with a rhodopsin minigene in cell culture leads to inhibition of pre-mRNA splicing of rhodopsin intron 3 [55]. Interestingly, a homozygous rhodopsin splice site mutation identified in RP patients affects the removal of this intron [93]. The observation that mutations in PRPF31 gene affect RHO pre-mRNA splicing suggests a functional link between PRPF31 and RHO, two major adRP genes [55]. The expression of the mutant PRPF31 protein inhibits RHO pre-mRNA splicing in the presence of wild-type protein supporting a potential dominant negative mechanism. Analyses of two missense mutations in the Nop domain, Ala216Pro and Ala194Glu, suggest that protein translocation into the nucleus is affected [94]. When mislocalized, such mutant proteins may also cause gain-of-function toxicity.

These potential mechanisms are not exclusive of each other. It is possible that different mutations in different genes cause adRP via distinct mechanisms. The final common outcome is the death of photoreceptor cells.

Conclusion: The combination of genetic, molecular, and biochemical studies have advanced our understanding of pathogenic mechanisms underlying inherited retinal degeneration. Recent progress in identification of adRP genes involved in pre-mRNA splicing, PRPF31, PRPF8, PRPF3, and PAP1, high-

lights the importance of post-transcriptional RNA processing, especially pre-mRNA splicing, in maintaining normal function of photoreceptors, and in the pathogenesis of retinal degeneration. The discovery that mutations in these adRP-linked pre-mRNA splicing factor genes result in photoreceptor loss indicates that photoreceptor cells may have unique properties that render them particularly sensitive to mutations in pre-mRNA splicing genes. This also suggests the existence of a common pathway by which pre-mRNA splicing defects lead to photoreceptor cell death.

Many questions remain to be addressed. It is not known what mechanism(s) initiate photoreceptor degeneration in patients carrying adRP-linked splicing gene defects. Most of such patients have relatively normal retina during early stages of their lives, indicating that the photoreceptor development is not affected by these adRP gene mutations. It is not clear which target genes are affected by such adRP-associated splicing gene mutations and whether these target genes share similar or distinct mechanisms in affecting photoreceptor survival and function. Identification of the downstream target genes of these adRP-associated pre-mRNA splicing factors, especially the photoreceptor-specific target genes, will help us understand the underlying molecular mechanisms and may lead to discovery of new pathways for retinal degeneration.

Both RP11 and RP9 show incomplete penetrance, suggesting a potential role of genetic modifier gene(s) in influencing the expression or activity of these adRP associated splicing factors. So far, no animal models have been reported for adRP associated splicing genes. It is possible that genetic background may also influence the phenotypic manifestations when such animal models are established.

Elucidating the pathogenetic mechanisms of adRP-linked pre-mRNA splicing factor mutations may also aid in the development of novel therapeutics for retinitis pigmentosa. If haploinsufficiency mechanism is responsible for the adRP defects, the primary aim should be restoration of the normal levels of wild-type proteins for the corresponding adRP-linked genes. On the other hand, if the dominant negative gain-of-function toxicity mechanism plays an important role in photoreceptor loss, then enhancing the clearance of the toxic mutant protein may be of therapeutic value if implemented early.

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